Isolation and Characterization of Spleen Acid Deoxyribonuclease

In recent years several methods have been described for the partial purification of spleen acid deoxyribonuclease (DNase)[1-4] (see also ref. 5 for a critical review). The isolation of the enzyme in a pure form was considered to be of interest, particularly in view of the results obtained in an examination of the kinetics of the enzymatic degradation of deoxyribonucleic acid[6]. The preparation procedure as well as a preliminary physical and chemical characterization of the enzyme are reported in the present communication.

The preparation of the enzyme was carried out at 4° C, using hog spleen as the starting material. In a first stage a crude enzymatic preparation was obtained essentially according to a procedure[7] involving the following steps: (1) homogenization and extraction of spleen tissue with 0·15 M sodium chloride–0·02 M calcium chloride; (2) precipitation of the clarified extract with ammonium sulphate; the precipitate collected between 34 and 100 per cent saturation was redissolved in distilled water; (3) acidification to *p*H 2·5, to precipitate hæmoglobin, and centrifugation; (4) precipitation of the supernatant from the preceding step with ammonium sulphate; the fraction obtained between 40 and 80 per cent saturation was dissolved in distilled water, clarified by centrifugation, freeze-dried, and stored at −30° C. The crude enzyme was obtained in a yield of about 1 g/kg tissue; it contained about 55 per cent of the acid DNase activity present in the clarified homogenate and its specific activity was 3·0. Both neutral and acid ribonuclease (RNase) activities[8] were present, the ratios DNase/RNase being 3·6 and 1·8, respectively. In all cases activities were measured by determining the optical density at 260 mμ of acid-soluble nucleotides[9] released by enzymatic action under suitable experimental conditions.

The second stage involved the following chromatographic steps: (1) *DEAE-cellulose:* the enzyme was washed out with 0·005 M phosphate buffer *p*H 8·0; the total activity was about 10 per cent higher after chromatography, probably because of the adsorption of an inhibitor on the column; the adsorbed protein, containing most of the RNase activity, could be eluted with 0·15 M acetate buffer, *p*H 5·0; (2) *hydroxyapatite:* elution was carried out with a linear gradient of phosphate buffer *p*H 6·8, of molarity increasing from 0·05 to 0·5; the enzyme was

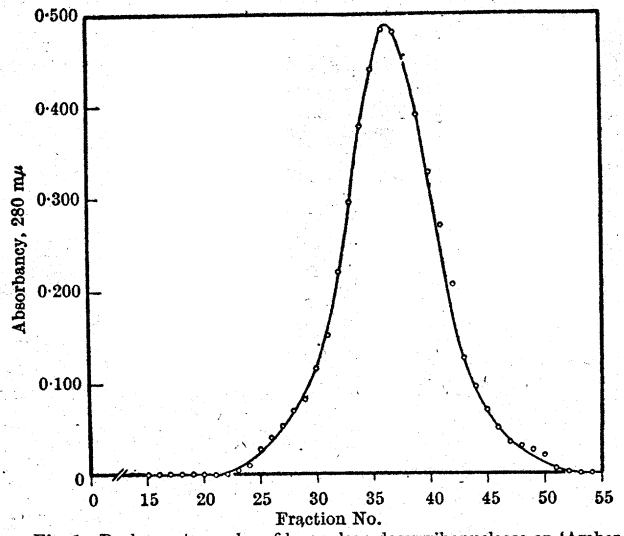

Fig. 1. Rechromatography of hog spleen deoxyribonuclease on 'Amberlite *IRC*-50' (2 cm × 12·5 cm). Elution was carried out at 4° C with a gradient of phosphate buffer *p*H 6·0, 0·1–0·5 M. No optical density was eluted with a subsequent gradient of 0·5 M phosphate buffer from *p*H 6·0 to 8·0. Fractions of 5 ml. were collected

eluted immediately after a bright red fraction, tentatively identified with cytochrome *c*; (3) *hydroxyapatite*: elution was performed as above, the enzyme being removed from the column at about 0·2 M phosphate; (4) '*Amberlite IRC–50*': the enzyme was loaded in 0·1 M phosphate buffer *p*H 6·0; some inactive protein was not retained on the column; the enzyme was eluted with a linear gradient of phosphate buffer *p*H 6·0, 0·1–0·5 M; a subsequent gradient of 0·5 M phosphate buffer *p*H 6·0–8·0 eluted more inactive protein. Rechromatography of the enzyme on 'Amberlite *IRC–50*' gave a symmetrical peak of constant specific activity equal to 210 ± 10 (Fig. 1); no acid or neutral RNase activity was detectable in this product. About 25 per cent of the enzymatic activity present in the crude enzyme was recovered after the final rechromatography.

An ultracentrifuge analysis showed that the enzyme sedimented as a single boundary in 0·15 M acetate buffer *p*H 5·0 (Fig. 2). No dependence on concentration was observed and the sedimentation coefficient was $S^0_{20,w} = 3{\cdot}4\ S$. The same value was obtained in acetate buffer *p*H 3·0, μ0·1, and phosphate buffer *p*H 7·8, μ0·1. In glycine buffer *p*H 8·9, μ0·1, the sedimentation coefficient was found to be 4·0 *S*, and a small amount of a faster component was present. In glycine buffer *p*H 2·0, μ0·1, the sedimentation analysis indicated extensive aggregation.

Electrophoretic runs were performed on cellulose acetate strips at 6 V/cm. Only a single band was evident

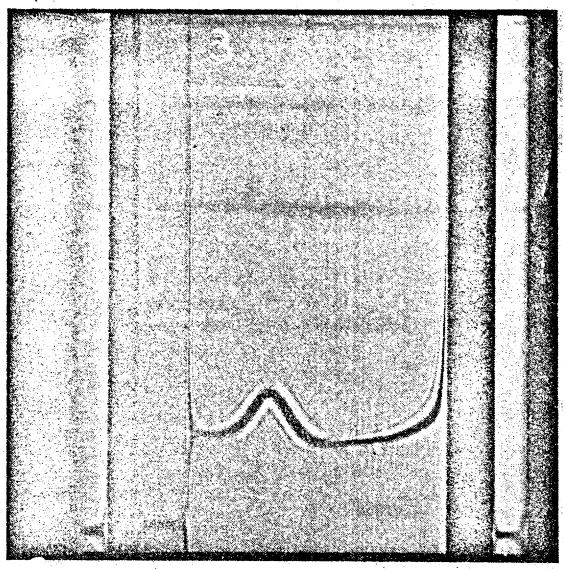

Fig. 2. Sedimentation pattern of hog spleen deoxyribonuclease in 0·15 M acetate *p*H 5·0. Exposure taken after 56 min at 59,780 r.p.m.

at four different *p*H values ranging from 4·6 to 9·2. By extrapolating the mobilities obtained as a function of *p*H to zero mobility, an isoelectric point close to 10·2 could be calculated.

An amino-acid analysis was carried out, using three different times of hydrolysis. Neutral and acidic amino-acids were predominant; the amount of ammonia (as estimated after extrapolation to zero time of hydrolysis) was consistent with most or all of the acidic amino-acids being in the form of the corresponding amides, a fact which would explain the high isoelectric point.

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